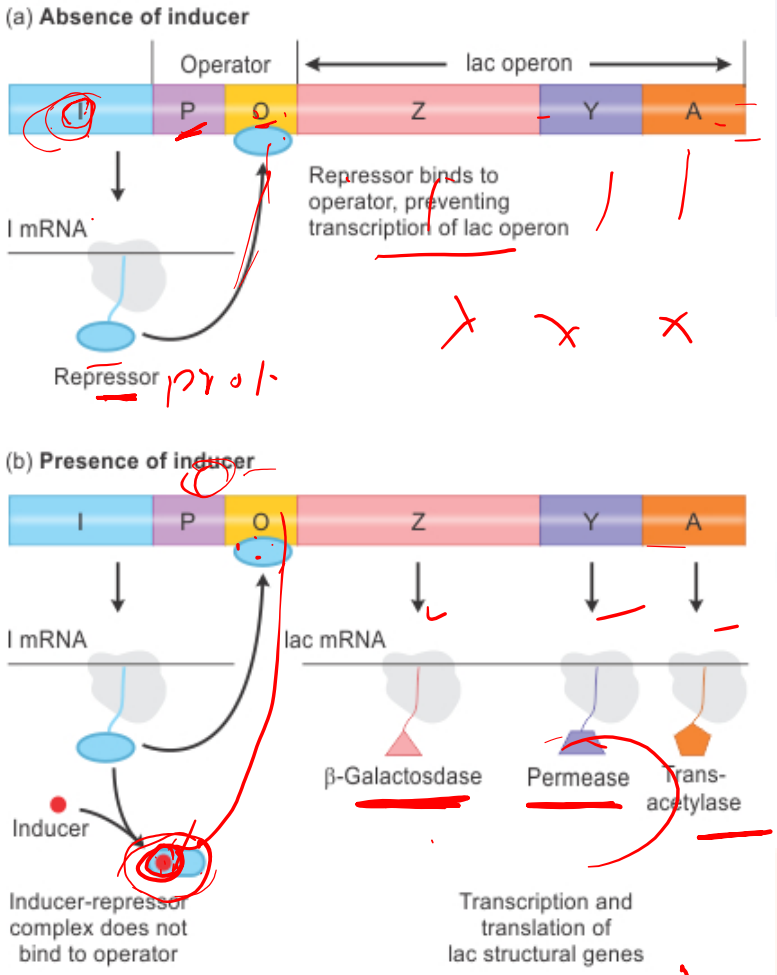
LAC- OPERON



i = inhibitor protein synthesizing gene

O - operator

P = promoter

Z, Y a - structural gene

Lactose metabolism

Lactose → Allolactose

→ 99 glu + galactose

WHAT IS HUMAN GENOME PROJECT ?

- **Human Genome Project (HGP) was called a mega project.**
- **It was an International effort formally begun in October, 1990.**
- **The HGP was a 13-year project coordinated by the**

↳ 2003

↳ 2006 (But was completed)

1990

U.S. DEPARTMENT OF ENERGY

&

NIH

National Institutes of Health

- **During the early years of the HGP, the Wellcome Trust (U.K.) became a major partner, and additional contributions came from Japan, France, Germany, China and others.**

- **The project was almost completed in 2003.**

Over 12 countries also participated in this project

- **Knowledge about the effects of DNA variations among individuals can lead to revolutionary new ways to diagnose, treat and someday prevent the thousands of disorders that affect human beings.**

- **HGP was closely associated with the rapid development of a new area in biology called Bioinformatics.**

HGP Mega project (Biology with computer)

1990

13 years

2003

3×10^9 bases

1 Base - $3

9 billion $

Bioinformatics

Standards

GOALS OF HGP:

i) To identify all the approximately 30,000 genes in human DNA.

ii) To determine the sequences of the 3 billion chemical base pairs that make up human DNA.

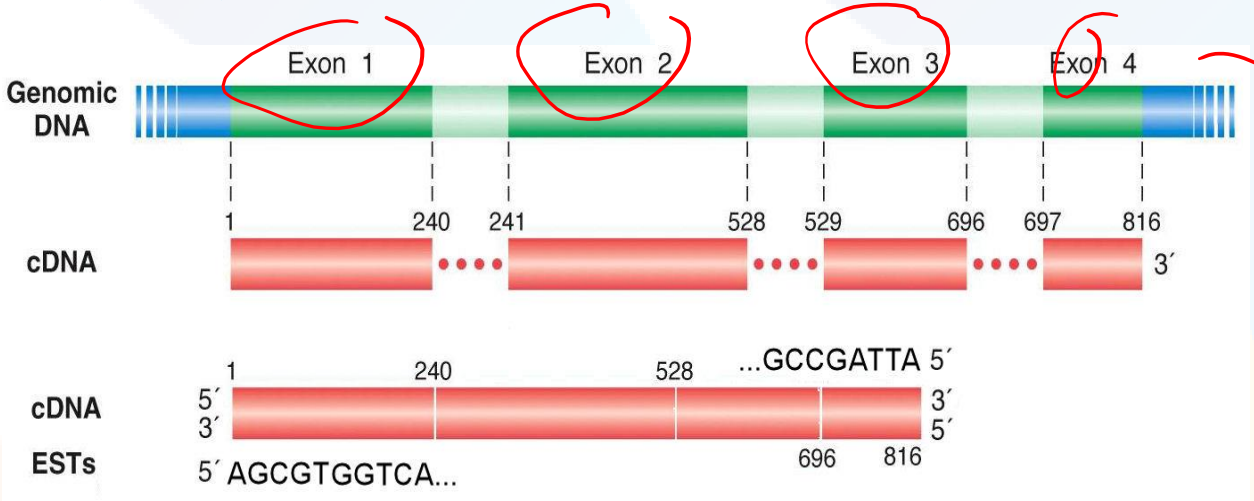
iii) To improve tools for data analysis.

iv) To address the ethical, legal, and social issues (ELSI) that may arise from the project.

METHODOLOGIES

The methods involved two major approaches.

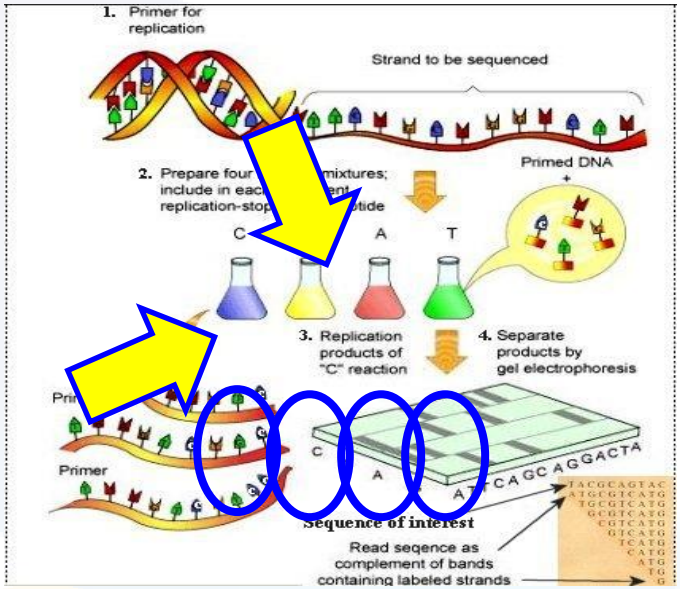
- **One approach focussed on identifying all the genes that expressed as RNA (referred to as Expressed Sequence Tags (ESTs).**



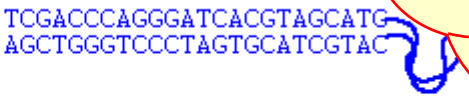
- **The other took the blind approach of simply sequencing the whole set of genome that contained all the coding and non-coding sequence, and later assigning different regions in the sequence with functions (a term referred to as Sequence Annotation).**

DNA SEQUENCING

DNA sequencing is the process of determining the exact order of the 3 billion paired chemical building blocks (called 'bases' - A, T, C, and G) that make up the DNA of the *24 different human chromosomes (23 + Y in a male)*



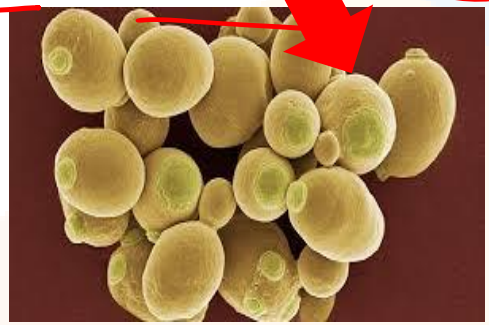
- **DNA sequencing was the greatest technical challenge in the Human Genome Project.**



2 DNA fragments

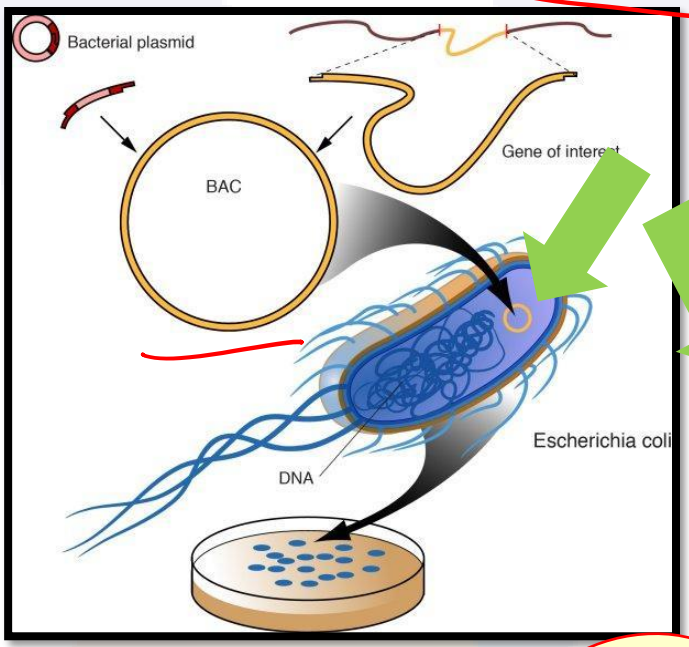
The cloning results in the amplification of DNA fragments, which are used for sequencing the bases.

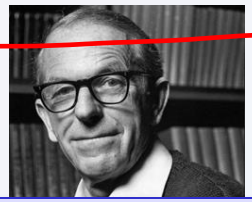
Commonly used hosts : Bacteria and yeast



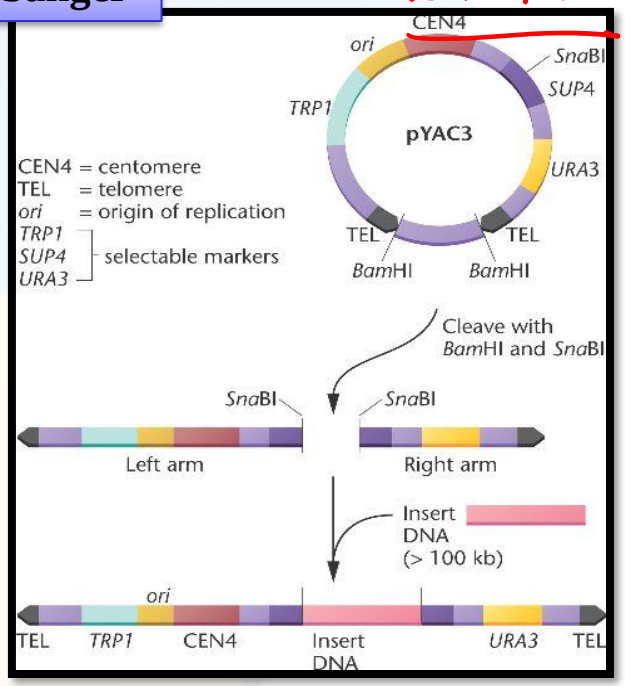
Commonly used *vectors* : BAC (bacterial artificial chromosomes)
YAC (yeast artificial chromosomes).

HGP
Can account date now DNA



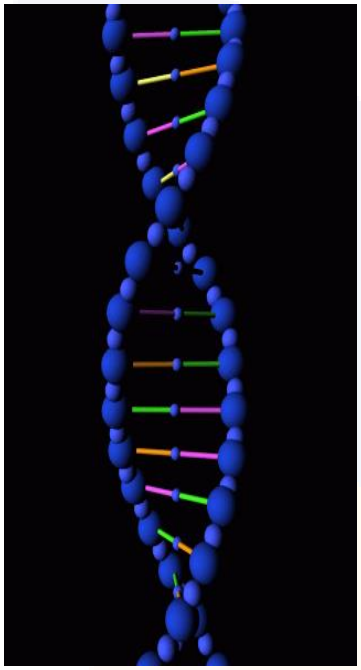


Frederick Sanger



The fragments were sequenced using automated DNA Sequencers that worked on the principle of a method developed by

SALIENT FEATURES OF HUMAN GENOME



i) The human genome consists of chromosomes made up of nucleotide base pairs.

ii) The average gene consists of 3000 bases, but sizes vary greatly, with the largest known human gene being the one that codes for the protein called dystrophin.

iii) The total number of genes is estimated at 30,000. Almost all (99.9 %) nucleotide bases are exactly the same in all people.

iv) The functions are unknown for over 50 % of the genes discovered.

v) Less than 2 per cent of the genome codes for proteins.

vi) Repeated sequences make up very large portion of the human genome.

vii) Repetitive sequences are stretches of DNA sequences that are repeated many times. They are thought to have no direct coding functions, but they shed light on chromosome structure, dynamics and evolution.

→ Satellite DNA

VNTR ← Microsatell Minisatell.

viii) Chromosome 1 has the highest number of genes (2,968). Y-chromosome has the fewest genes (231).

ix) Scientists have identified about 1.4 million locations where single base DNA differences occur in humans.

This information promises to revolutionise the processes of finding chromosomal locations. For disease-associated sequences and tracing human history.

SNPs – single nucleotide polymorphism, pronounced as snips

ADVANTAGES OF HGP

- **In the area of health care, identification and mapping of the genes responsible for genetic diseases help in diagnosis, treatment and prevention of these diseases.**

- **Detailed knowledge of the genomes of humans and other species will give a clearer picture of Gene expression, Cellular growth and differentiation and evolutionary biology.**

ADVANTAGES OF HGP

- **Earlier detection of genetic predispositions to disease, rational drug design, Gene therapy was going to be easy with more knowledge on human genome.**

- **A new era of Molecular Medicine, characterized by looking into the most fundamental causes of disease than treating the symptoms will be an important advantage.**

Over 99% of the 3 billion nucleotide pairs in human DNA are identical among all individuals.

No two people (other than identical twins) have exactly the same sequence of bases in their DNA.

Restriction Fragment Length Polymorphisms are characteristics to every person's DNA.

RFLPs are pronounced as riflips

Restriction Fragment Length Polymorphisms are called Variable Number Tandem Repeats (VNTRs)

The VNTRs of two persons generally show variations.

DNA fingerprinting involves identifying differences in some specific regions in DNA sequence called repetitive DNA, because in these sequences, a small stretch of DNA is repeated many times.

RFLPs or VNTRs are useful as Genetic markers.

For example in the following hypothetical example nucleotide base sequence, there are 6 Tandem Repeats of 16 bases each (count the first 16 and note how they are repeated)

5’ GACTGCCTGCTAAGATGACTGCCTGCTAAGATGACTGCCTGCTAAGAT
GACTGCCTGCTAAGATGACTGCCTGCTAAGATGACTGCCTGCTAAGAT3’

G

A

C

T

Such clusters of 10 -100 nucleotides are called mini satellites.

Such tandem repeats are characteristic of every person’s DNA.

The VNTRs of two persons differ in the number of tandem repeats or the sequence of bases.

Such changes are caused due to *mutations* and *gene recombinations*.

Example : A child might inherit a chromosome with 6 tandem repeats from the mother and the same tandem repeated 4 times from the father in a homologous chromosome.

It means half of the VNTR alleles of the child resemble those of the mother and the other half those of the father.

This is a 'heterozygous condition with reference to VNTR alleles'.

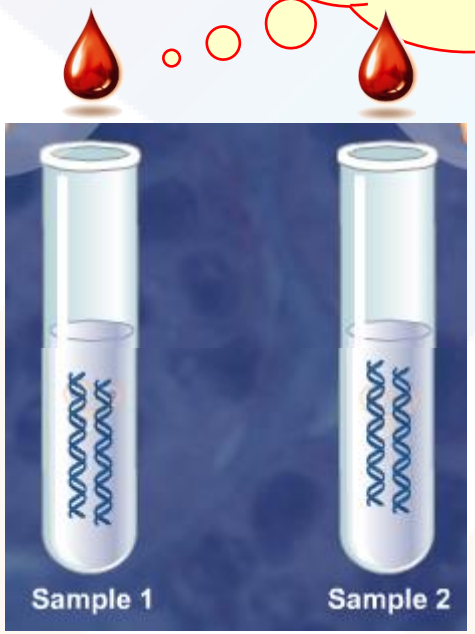
Tandem repeats serve as basis of the technique, DNA fingerprinting.

DNA fingerprinting is a technique by which the DNA of an individual can be compared with that found in a sample or another individual (a suspect in a crime).

DNA Isolation

DNA obtained from blood, semen, hair root sample, etc.

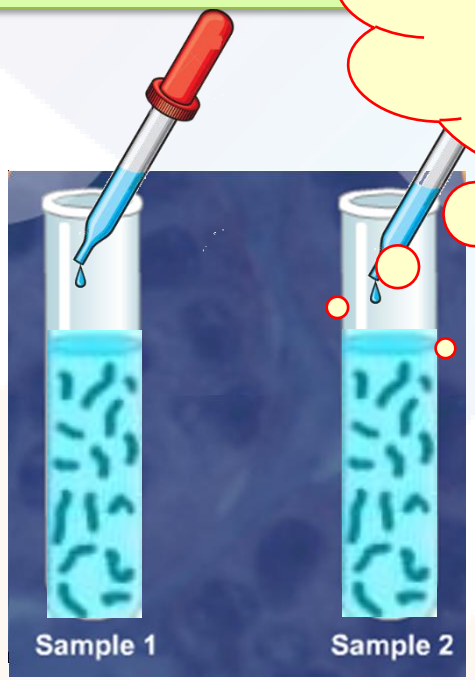
The technique is carried out as follows



Polymerase Chain Reaction

If DNA obtained is very less in quantity then it is <u>amplified</u> using PCR

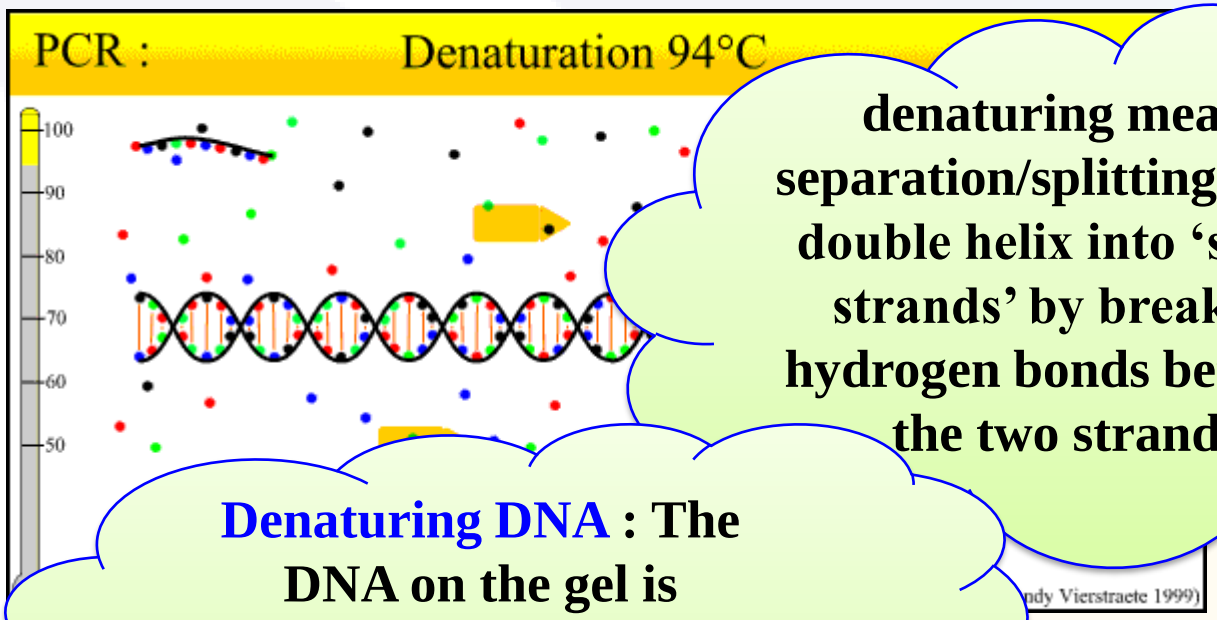
DNA fragmentation



DNA sample is fragmented using restriction endonucle

Many fragments are obtained having variable length

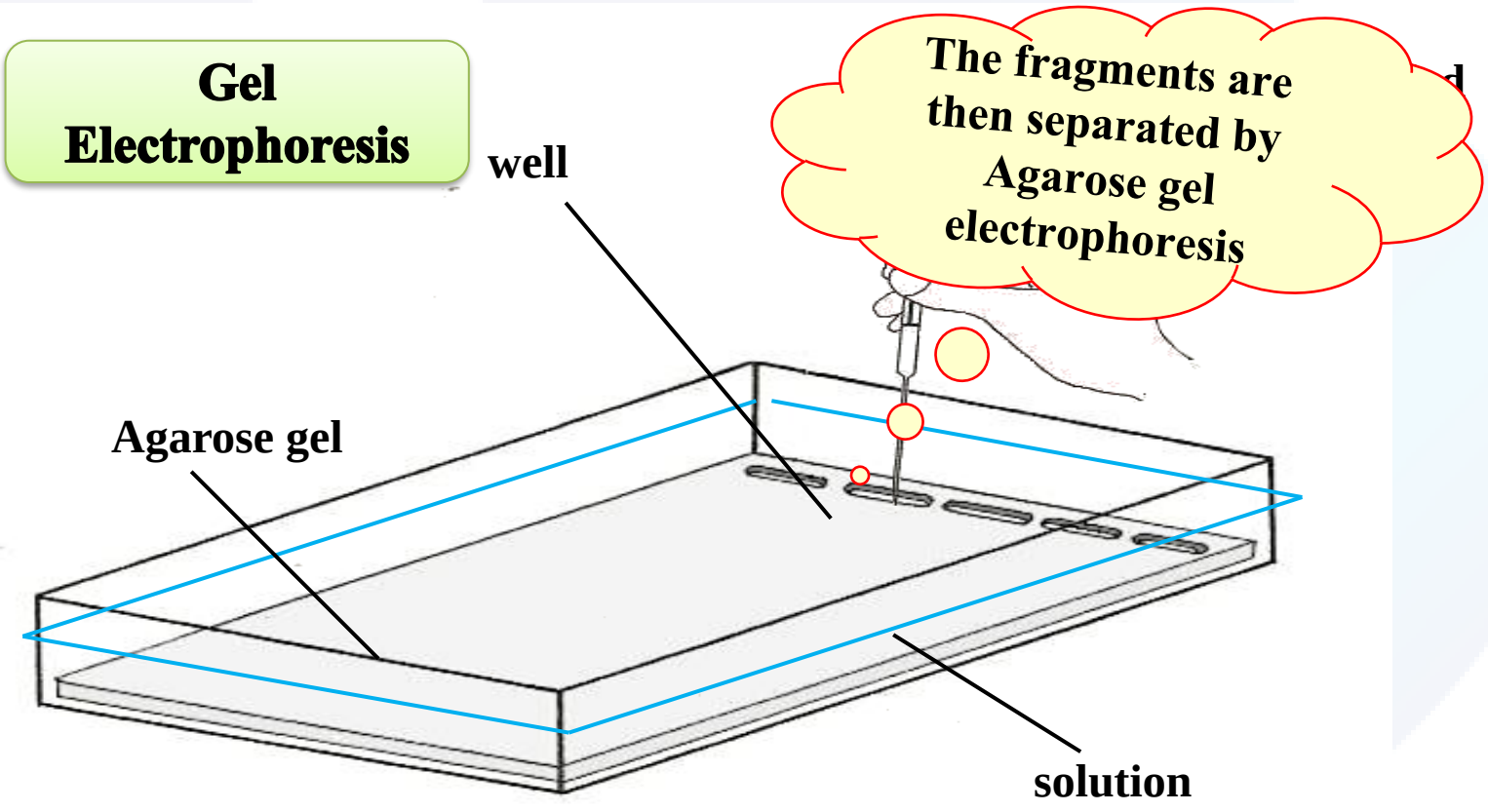
Restriction Fragment Length Polymorphism (RFLP)

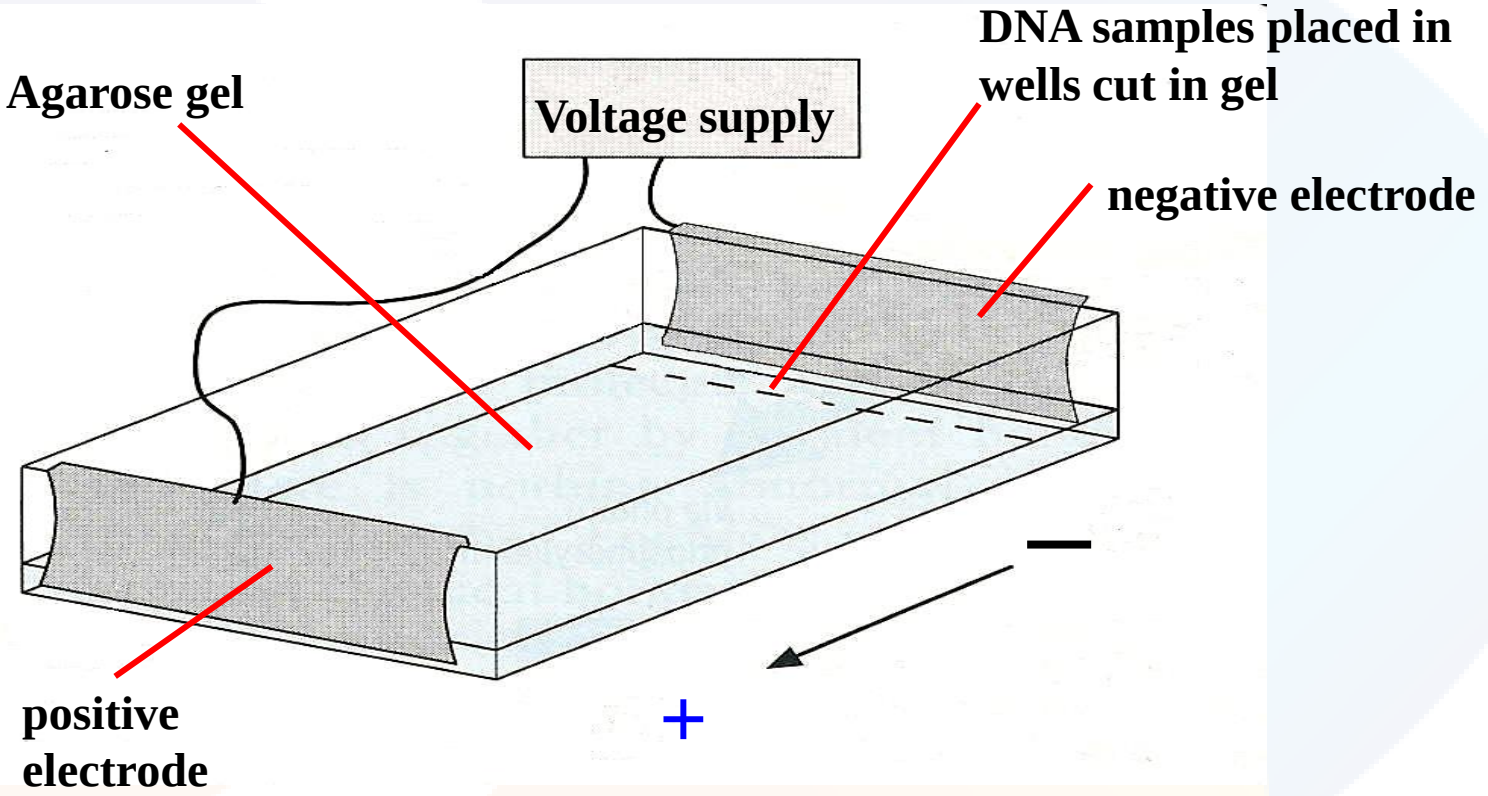


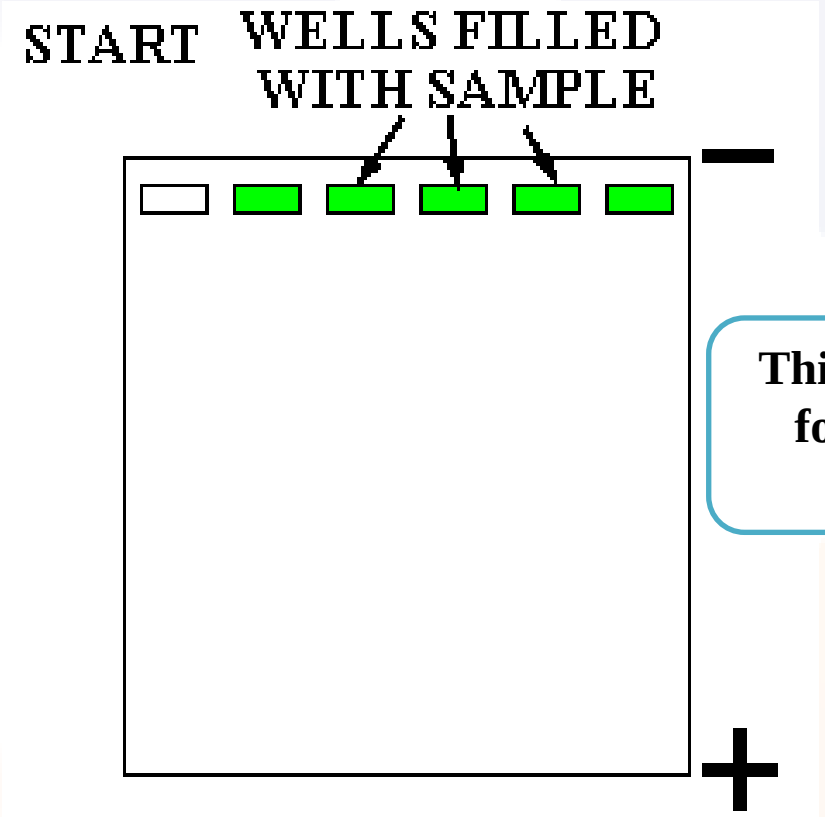
denaturing means separation/splitting of the double helix into 'single strands' by breaking hydrogen bonds between the two strands

Denaturing DNA : The DNA on the gel is 'denatured' using alkaline chemicals or by heating.

Gel Electrophoresis

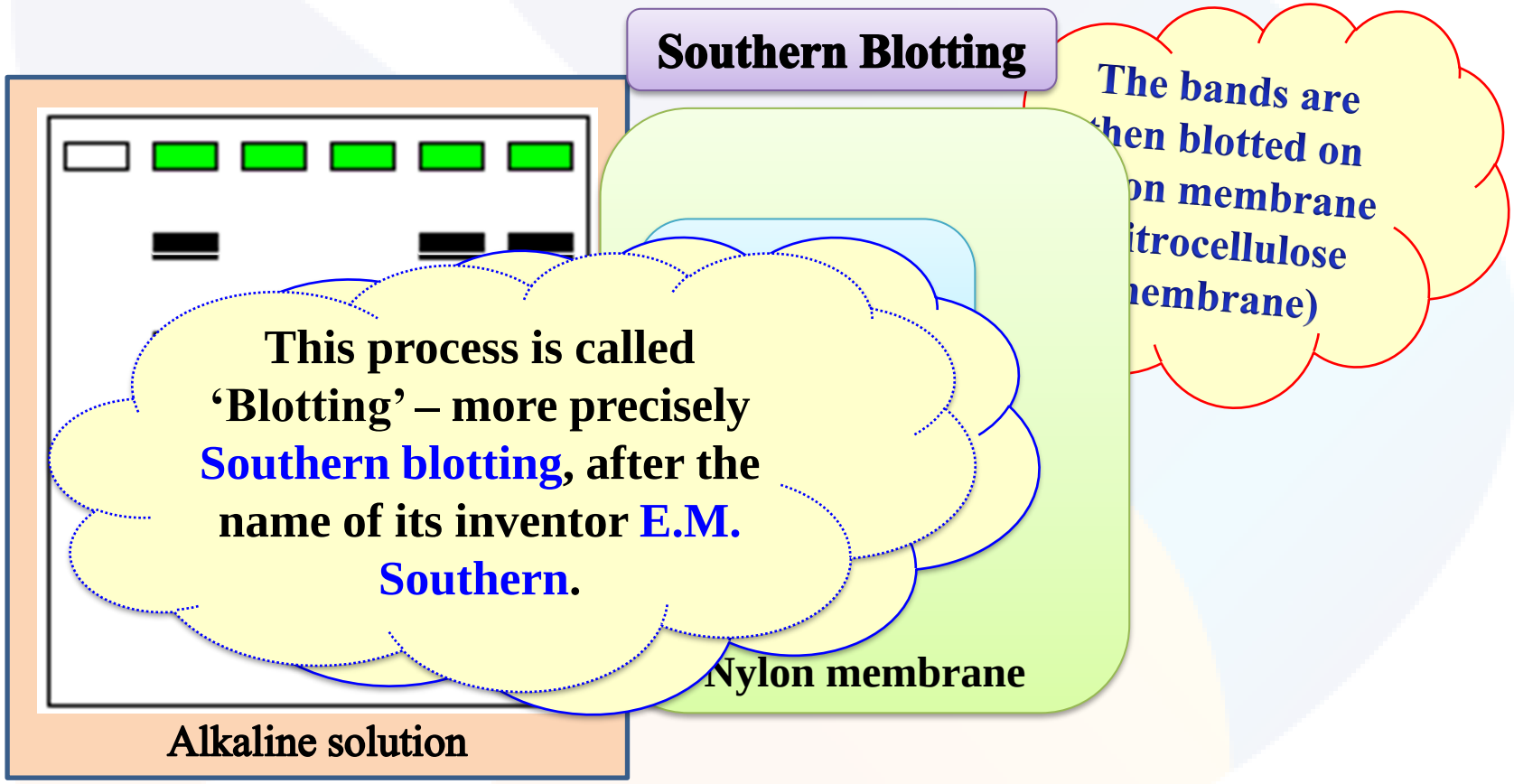


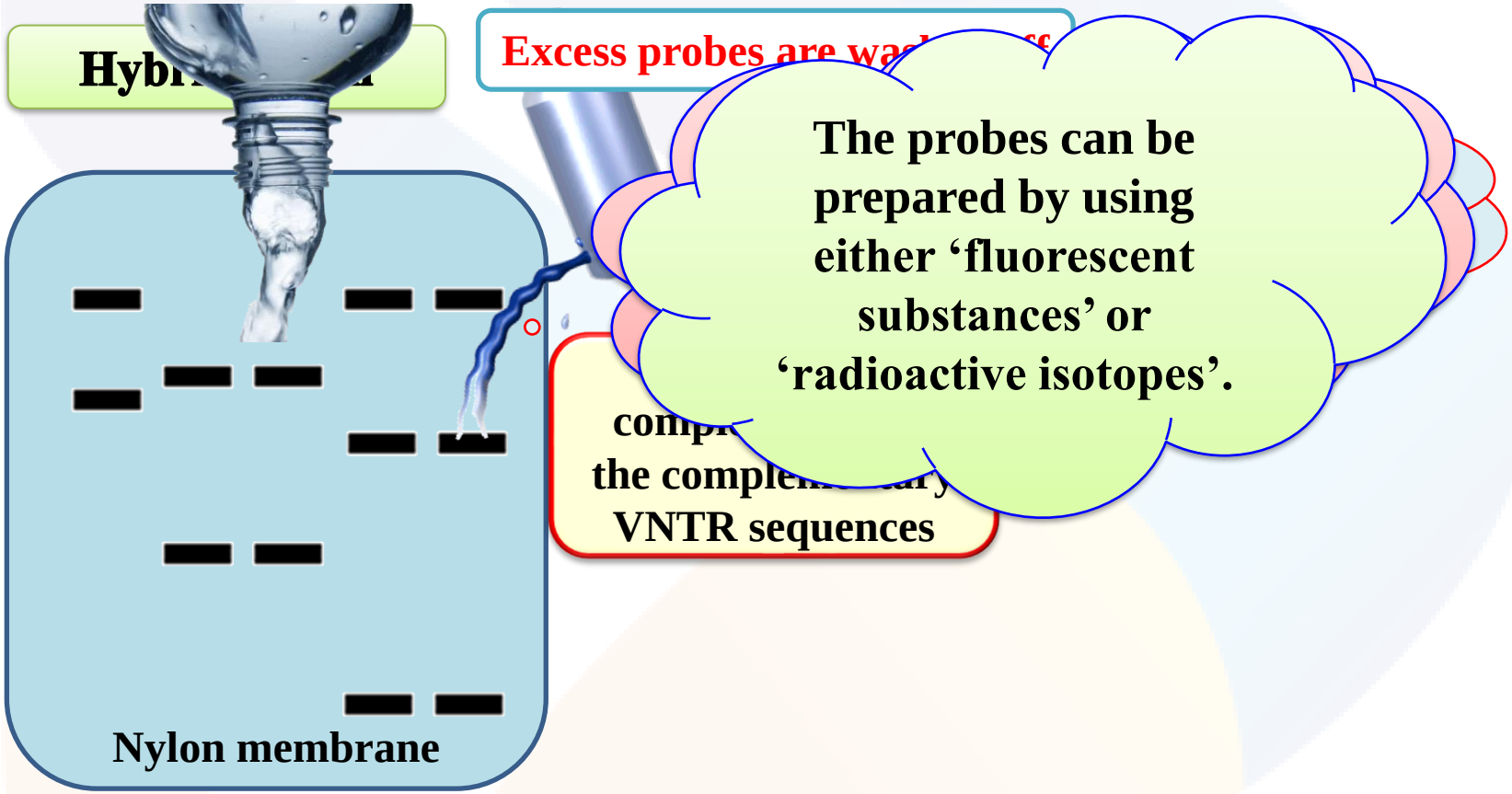

Agarose gel
Voltage supply
DNA samples placed in wells cut in gel
negative electrode
positive electrode
–
+



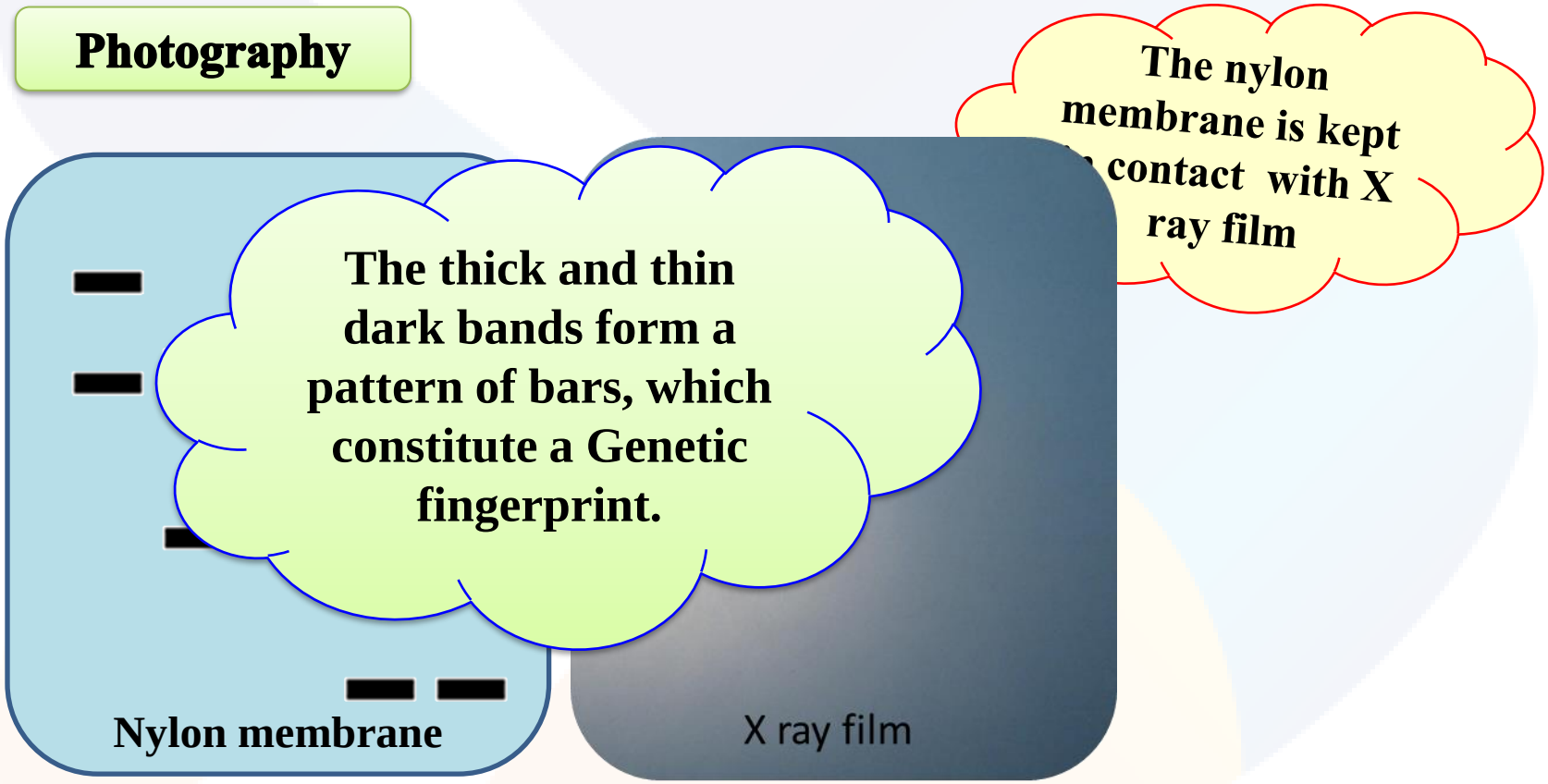
The movement of these fragments depend on their length

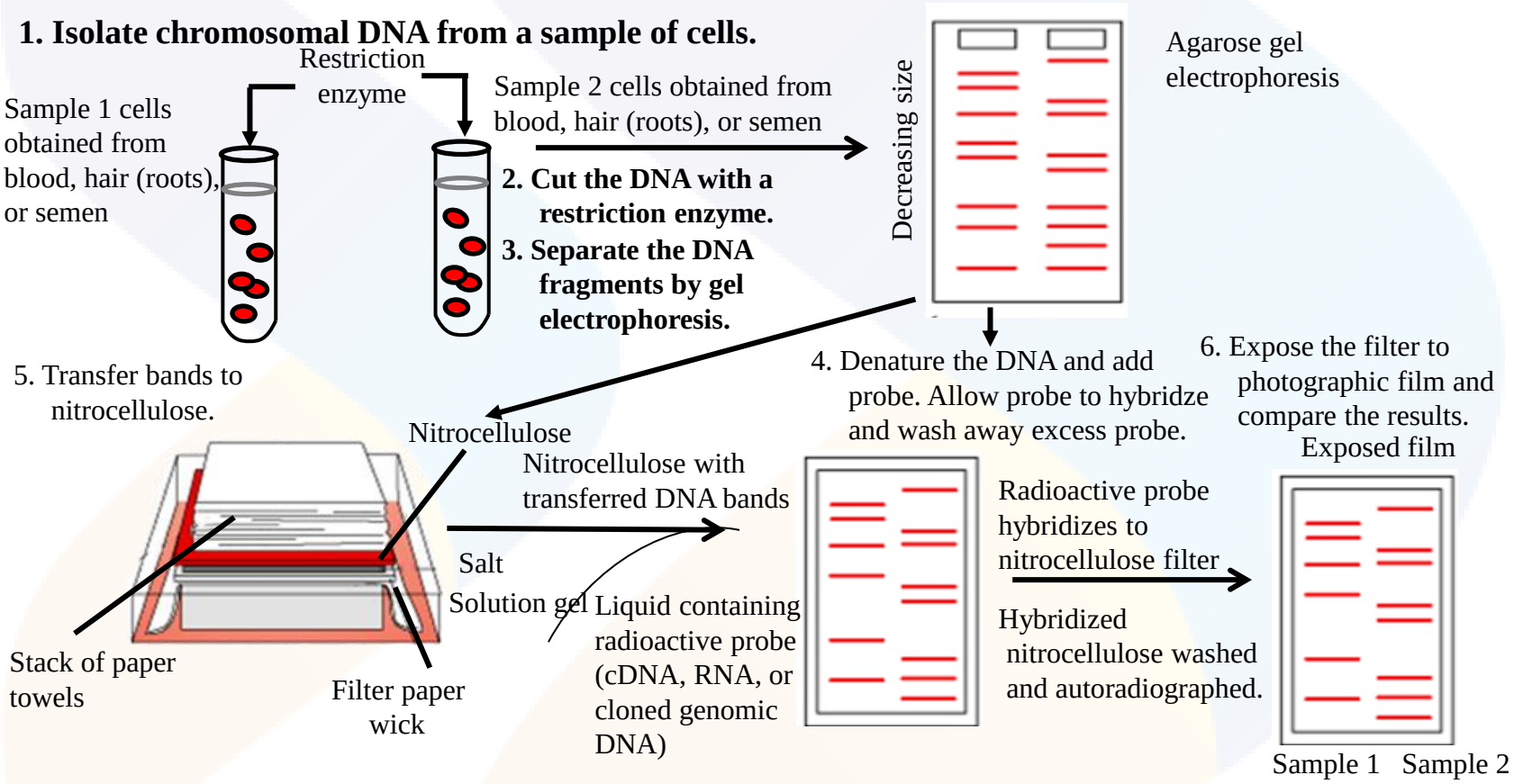
This results into formation of <u>bands</u>


Southern Blotting
The bands are
hen blotted on
on membrane
itrocellulose
embrane)
This process is called
'Blotting' – more precisely
Southern blotting, after the
name of its inventor E.M.
Southern.
Nylon membrane
Alkaline solution


Hybr
Excess probes are wa
The probes can be prepared by using either ‘fluorescent substances’ or ‘radioactive isotopes’.
compl
the complem ary
VNTR sequences
Nylon membrane

Photography




1. Isolate chromosomal DNA from a sample of cells.
Restriction enzyme
Sample 1 cells obtained from blood, hair (roots), or semen
Sample 2 cells obtained from blood, hair (roots), or semen
2. Cut the DNA with a restriction enzyme.
3. Separate the DNA fragments by gel electrophoresis.
Decreasing size
Agarose gel electrophoresis
4. Denature the DNA and add probe. Allow probe to hybridze and wash away excess probe.
5. Transfer bands to nitrocellulose.
Nitrocellulose
Nitrocellulose with transferred DNA bands
Salt Solution gel
Stack of paper towels
Filter paper wick
Liquid containing radioactive probe (cDNA, RNA, or cloned genomic DNA)
Radioactive probe hybridizes to nitrocellulose filter
Hybridized nitrocellulose washed and autoradiographed.
6. Expose the filter to photographic film and compare the results.
Exposed film
Sample 1
Sample 2

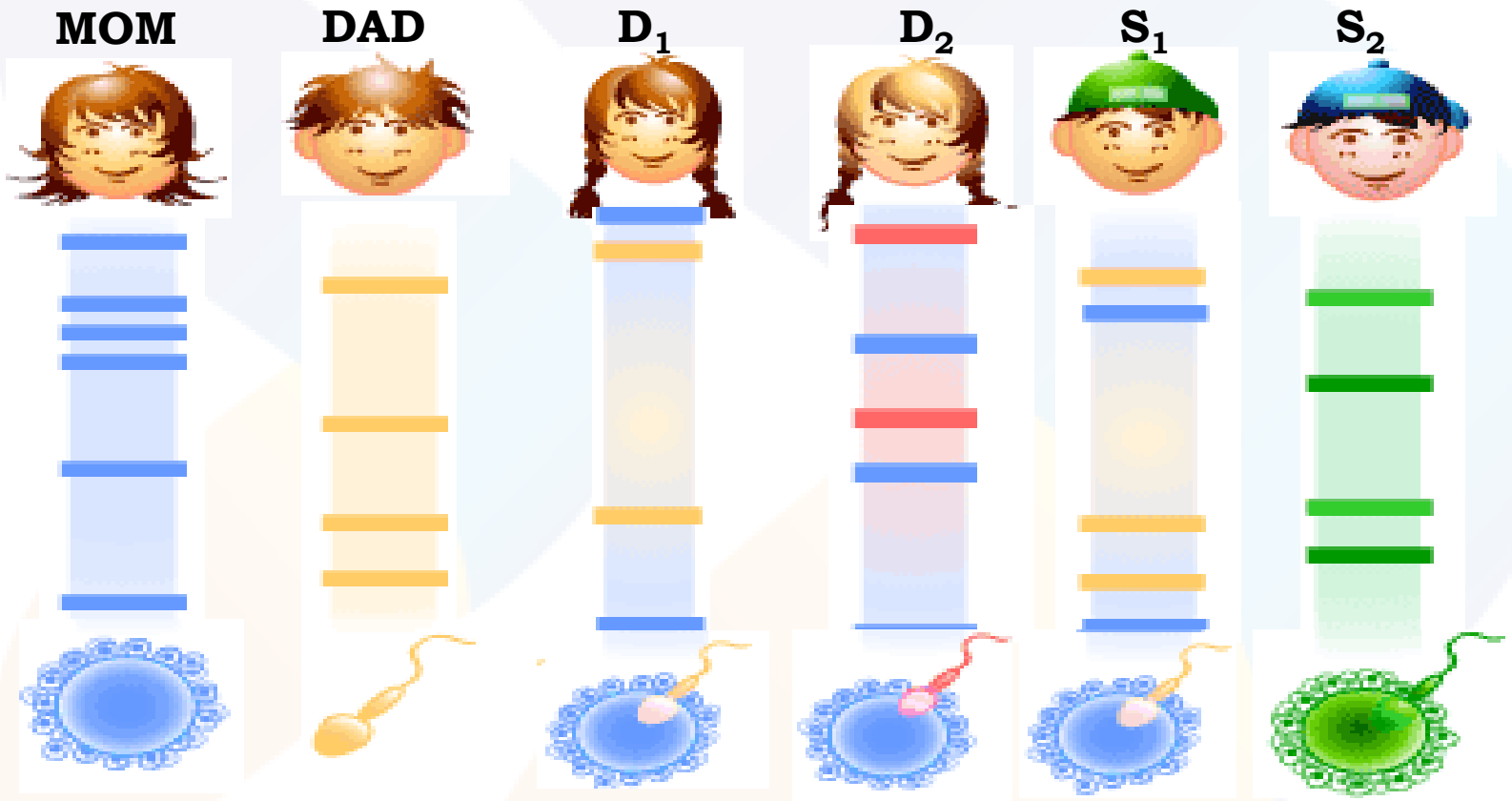
Obtaining hybrid with radioactive probe and matching DNAs of different members of a family with biological children and adopted children, gives us an idea of how DNA Finger Prints help identification of paternity/maternity, by studying the 'DNA Finger Prints' of members of a Family – Biological and non-biological relationships.

A given person can never have a VNTR, which his parents do not have

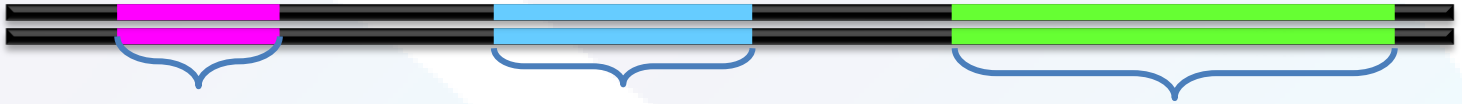
The illustrations given below are the VNTR patterns for

- ✓ **Mrs. Rose [blue]**
- ✓ **Mr. Rao [yellow]**
- ✓ **D1 (Mr. Rao's bio**
- ✓ **D2 (Child of Mrs. Rose and her for husband)**
- ✓ **S1 (Mr. Rao's biological so)**
- ✓ **S2 (Mr. Rao's adopted son**

not biologically related, his parents' DNA marked in light and dark green bands.


MOM
DAD
D1
D2
S1
S2

1. Section of victim's DNA :



24 repeats of 'GT'

16 repeats of 'ATGG'

66 repeats of 'ATT'

There are three regions of repetitive DNA.

49 repeats of ‘GT’

16 repeats of ‘ATGG’

51 repeats of ‘ATT’

The same three regions of repetitive DNA are present here, but some include different numbers of repeats. Now let’s compare this sample to……

3. Section of DNA from crime scene hair :

49 repeats of 'GT'

16 repeats of 'ATGG'

51 repeats of 'ATT'

The length of the repetitive sequences match the lengths in the suspect's DNA – so the DNA found at the crime scene belongs to the suspect.

APPLICATIONS OF DNA FINGER PRINTING

- **Conservation of wild life – protection of endangered species. By maintaining their DNA records for identification of tissues of the dead endangered organisms**
- **Taxonomical applications – study of phylogeny.**
- **Anthropological studies – charting of origin and migration of human population.**
- **Pedigree analysis – inheritance pattern of gene through generations.**
- **Medico-legal cases – establishing paternity and/or maternity more accurately.**
- **Forensic analysis – positive identification of a suspect in a crime.**